

***In vitro* and *in vivo* synthesis of the hepatitis B virus surface antigen and of the receptor for polymerized human serum albumin from recombinant human adenoviruses**

Annick Ballay¹, Massimo Levrero^{1,2}, Marie-Annick Buendia¹, Pierre Tiollais² and Michel Perricaudet¹

¹Institut de Recherches Scientifiques sur le Cancer, Centre National de la Recherche Scientifique, 7, rue Guy Moquet, 94800 Villejuif, and ²Unité de Recombinaison et Expression Génétique, (INSERM U.163, CNRS LA 271), Institut Pasteur, 28, rue du Docteur Roux, 75724 Paris Cedex 15, France

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We have developed an adenovirus vector to express foreign proteins under the control of the adenovirus E1a promoter. Two recombinant plasmids, harbouring either the S gene or the pre-S2 region and the S gene of hepatitis B virus under the control of the E1a promoter, were used to construct two recombinant adenoviruses. These two viruses direct the synthesis of hepatitis B virus surface antigen (HBsAg) particles during the time course of an infectious cycle. When the pre-S2 region is present in the constructed virus, the synthesis of particles carrying the receptor for polymerized human serum albumin (pHSA) is observed. Moreover, the inoculation of rabbits with this latter purified recombinant adenovirus elicits the production of antibodies that react with both HBsAg and pHSA receptor.

Key words: eukaryotic expression vector/hybrid adenovirus/E1a promoter/hepatitis B virus/Vero cells

Introduction

Several viral vectors allowing propagation and expression of a foreign gene in mammalian cells have been developed (Elder *et al.*, 1981; Gluzman, 1982; Rigby, 1982). The use of human adenovirus (Ad) as a vector presents several advantages: (i) the understanding of the genetic organization of human adenoviruses type 2 or 5 (Ad2, Ad5) allows the viral genome to be manipulated in order to place a foreign gene under the control of an Ad promoter; (ii) a recombinant Ad harbouring up to 7 kbp of foreign DNA can efficiently be amplified on Ad-transformed human cell lines; (iii) the elevated copy number of the Ad genome during viral replication and the strong efficiency of the Ad promoters permit an overproduction of Ad-specific mRNAs; and (iv) the propagation of Ad in humans allows this virus to be considered as a potential vector to express a cloned gene *in vivo* in man.

Hepatitis B surface antigen (HBsAg) is one of the most suitable markers to test the efficiency of an expression vector in mammalian cells. HBsAg synthesized in animal cells is glycosylated, assembled and secreted into the cell supernatant. HBsAg gene expression is therefore very easy to detect by radioimmunoassay (RIA). *In vivo*, HBsAg synthesis induces the appearance of antibodies to HBsAg (anti-HBs), making easy the detection of HBsAg gene expression in animals.

We have constructed a plasmid which allows easy positioning of a coding sequence under the control of the early promoter of the E1a region of Ad5 (Van Ormondt *et al.*, 1978). The S gene and the pre-S2 region of hepatitis B virus (HBV) were inserted into this plasmid. The S gene codes for HBsAg and the

pre-S2 region for polymerized human serum albumin (pHSA) receptor (Heerman *et al.*, 1984; Strandring *et al.*, 1984). The subsequent construction of an Ad recombinant genome was made *in vitro* through the unique *Cla*I restriction site located at 2.6 mu (D'Halluin *et al.*, 1983). The recombinant Ad directs *in vitro* the synthesis of HBsAg particles carrying a pHSA receptor activity. Injected into rabbits, this recombinant virus elicits the production of anti-HBs and anti-pHSA receptor antibodies. This shows the feasibility of using recombinant adenovirus to express a gene both *in vitro* and *in vivo*.

Results

Construction of the expression plasmid pAB1

We started from a plasmid, pE1a (TaqI), which contains the very left end of Ad5 as a *Taq*I restriction fragment (nucleotides 1–631) cloned between the *Eco*RI and *Cla*I restriction sites of pML2 (Lusky and Botchan, 1981), (Figure 1A.) Both restriction sites are conserved during the cloning. This plasmid contains the promoter and the very beginning of the coding sequence of the E1a region. This latter part of E1a was subsequently removed by deleting the *Hae*III-*Pvu*II (495–623) restriction fragment. Plasmid pAB1 which results from this deletion possesses two unique restriction sites, *Cla*I and *Hind*III, located near the initiation site of transcription (Figure 1B). These restriction sites are particularly suitable for cloning restriction fragments to be transcribed from the E1a promoter.

Plasmid pAB1 contains a functional E1a promoter

To test the biological activity of the pAB1 plasmid, the *Bgl*II-*Bam*HI (nucleotides 5235–2533) restriction fragment of SV40 DNA was inserted between the *Hind*III and *Bam*HI restriction sites of pAB1 (Figure 1C). The resulting plasmid pK4 harbours the A gene which codes for SV40 T antigen downstream from the E1a promoter. Transient expression of T antigen was tested in HeLa cells after DNA-mediated transfection. About 5% of the cells transfected with pK4 were found to be T antigen positive by indirect immunofluorescence using a specific anti-T monoclonal antibody (O. Kellermann and F. Kelly, personal communication), whereas no synthesis was directed by a plasmid harbouring the SV40 fragment inserted in the wrong orientation. This shows that SV40 gene transcription is, in fact, under the control of the E1a promoter.

Use of pK4 to express HBV coding sequences

The coding region of the A gene of SV40 is bordered by two restriction sites, *Hind*III and *Bam*HI, which are respectively located 10 nucleotides upstream from the initiation codon and 70 nucleotides downstream from the polyadenylation signal AATAAA. Thus, these two sites in pK4 can be used to replace the coding region of the A gene of SV40 by any fragment of DNA containing the complete coding region and the 3'-untranslated region of a foreign gene. To test this possibility, we have inserted two different restriction fragments of the HBV genome between the *Hind*III and *Bam*HI restriction sites of pK4. One fragment maps between the *Xho*I and *Bgl*II restriction sites of

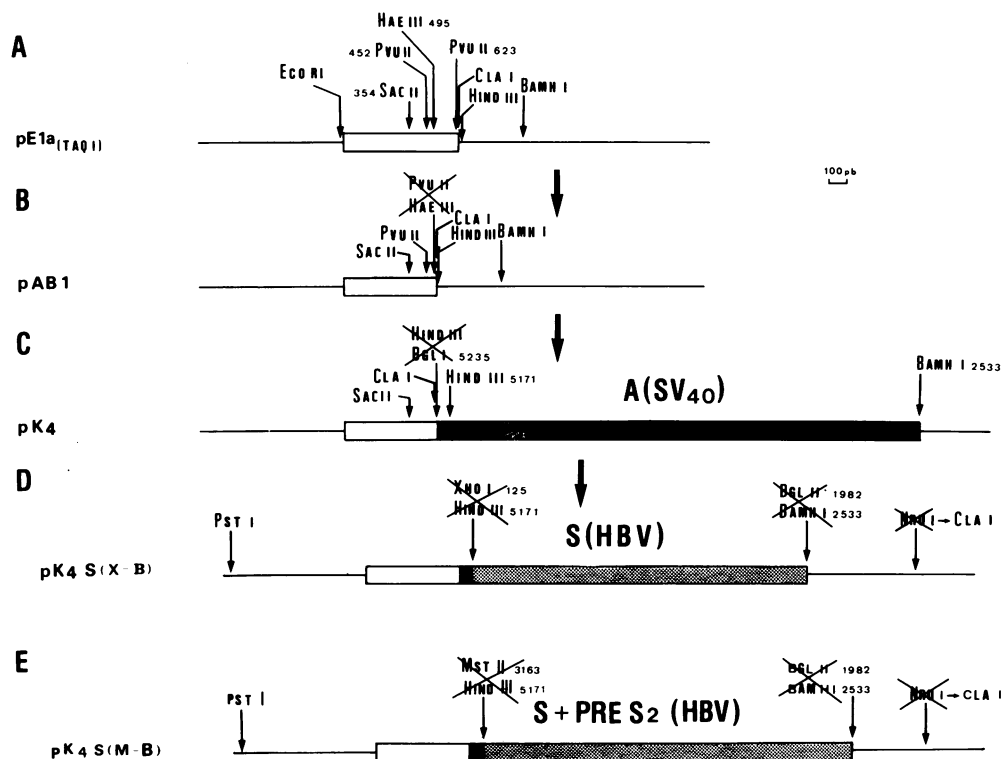


Fig. 1. Detailed structures of the plasmids used to construct the HBV-containing plasmids pK4 S(X-B) and pK4 S(M-B). Adenovirus sequences are indicated by open segments, SV40 sequences by black segments, HBV sequences by dotted segments and pBR322 sequences by lines. Numbers refer to the positions of restriction sites in the corresponding viral DNA fragments (Tiollais *et al.*, 1981; Tooze, 1981; Van Ormondt *et al.*, 1978).

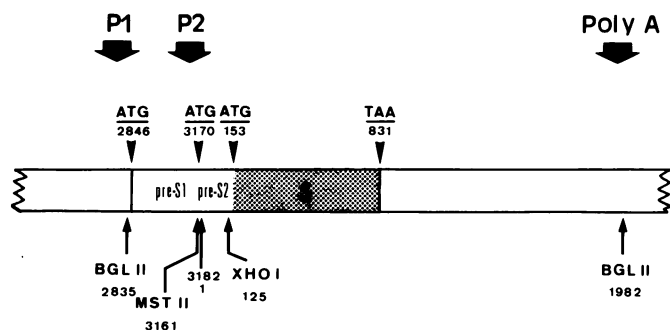


Fig. 2. Genetic map of the S gene and the pre-S1 and pre-S2 regions of the HBV genome. Two promoters P1 and P2 have been located upstream from the pre-S1 and pre-S2 regions. The poly(A) addition site precedes the *Bgl*II site (1982) by a few nucleotides. The S gene is represented by a dotted area, whereas the pre-S1 and pre-S2 regions are represented by an open area.

HBV (nucleotides 125–1982) and codes for the HBsAg (Figure 1D); the other one maps between the *Mst*II and *Bgl*III restriction sites (nucleotides 3161–1982) and codes for the HBsAg, as well as for the pHSA receptor (Figure 1E). The *Xho*I site precedes the initiation codon of the S gene by 28 nucleotides whereas the *Mst*II site precedes the initiation codon of the pre-S2 region by nine nucleotides. The *Bgl*III site is located 64 nucleotides downstream from the poly(A) addition signal of the S gene (Figure 2).

The capacity of these plasmids, pK4S(X-B) and pK4S(M-B), to direct the synthesis of HBsAg polypeptide and pHSA receptor was then tested after DNA-mediated transfection of Vero cells. HBsAg was detected in the cell culture medium by RIA. Both recombinant plasmids were found to direct the synthesis of authentic HBsAg polypeptides able to be assembled and excreted

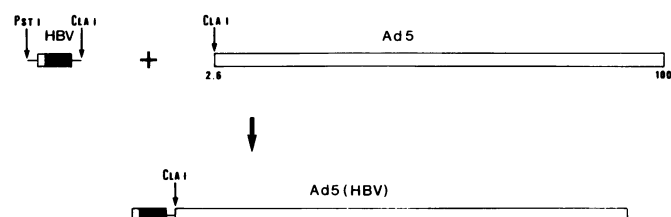


Fig. 3. Schematic construction of recombinant adenoviruses. The recombinant AdS(X-B) and AdS(M-B) viruses result from the ligation of the *Pst*I–*Cla*I restriction fragment of either pK4 S(X-B) or pK4 S(M-B) with the purified *Cla*I restriction fragment of Ad5 (2.6–100 mu). HBV sequences are depicted in black, while those of Ad5 are in white.

into the cell culture medium. Recombinant plasmids harbouring the HBV fragments in the wrong orientation with regard to the E1a promoter were unable to produce the antigen. This shows that the E1a promoter directs the transcription of the S gene into an mRNA which is efficiently and correctly translated.

Construction of recombinant adenoviruses

Plasmids pK4S(X-B) and pK4S(M-B) were modified by inserting a short DNA fragment containing a *Cla*I restriction site into the *Nru*I restriction site of pML2 (Figure 1D, E).

These modified plasmids were digested by *Pst*I and *Cla*I and the restriction fragments which contained the gene of interest were gel purified. The large *Cla*I restriction fragment of Ad5 (2.6–100 mu) was purified by sedimentation through a 5–40% sucrose gradient in Tris 100 mM, EDTA 100 mM, NaCl 1 M during 18 h at 29 000 r.p.m. The *Pst*I–*Cla*I restriction fragments isolated from plasmids (5 µg) were ligated with the sucrose gradient-purified Ad5 vector (20 µg). After ligation, the DNA was used to transfect Ad5-transformed human cells 293 (Aiello

Table I. Time course of extracellular HBsAg production

| Day | AdS(X-B) infected Vero | AdS(X-B) infected 293 | AdS(M-B) infected Vero | AdS(M-B) infected 293 |
|-----|------------------------------|-----------------------------|------------------------------|-----------------------------|
| 1 | 13 | 15 | 106 | 35 |
| 2 | 17 | 90 | ND | ND |
| 3 | 45 | 110 | 349 | 56 |
| 4 | 110 | 100 | 668 | 60 |
| 5 | 320 | 130 | 1057 | 63 |
| 6 | 470 | 120 | 1153 | ND |
| 7 | 550 | 120 | 900 | ND |

The cumulative amounts of HBsAg (expressed in ng) produced after infection of 10^6 293 cells and 10^6 Vero cells by either AdS(X-B) or AdS(M-B) are indicated.

et al., 1979; Graham *et al.*, 1977; Spector, 1983) using calcium phosphate precipitation. Two recombinant adenoviruses, AdS(X-B) and AdS(M-B), were isolated after 10 days (Figure 3).

Recombinant adenoviruses express authentic HBsAg

Levels of expression of the HBsAg synthesized after AdS(X-B) and AdS(M-B) infection of 293 and Vero cells are shown in Table I.

The kinetics of the intra and extracellular distribution of HBsAg from AdS(M-B)-infected Vero cells indicated that the HBsAg synthesis started within 3 h after infection and could be detected in the medium by 8 h (data not shown). Infection by the recombinant adenovirus AdS(M-B) at a multiplicity of infection of 10 p.f.u. per cell leads to an accumulation of HBsAg of 0.5–1 $\mu\text{g}/10^6$ cells in the medium after 120 h. Repeated experiments show that, in Vero cells, the recombinant adenovirus which contains the pre-S2 region synthesizes larger amounts of HBsAg than the recombinant which only contains the S gene.

Biophysical and biochemical characterization of the HBsAg synthesized by the recombinant adenoviruses

HBsAg purified from the cell culture medium of recombinant adenovirus AdS(M-B)-infected cells consisted of a homogeneous population of particles with a mean diameter of 22 nm as visualized by electron microscopy (data not shown). The density after centrifugation in CsCl was 1.21 g/cm³ (data not shown). Immunoprecipitation of ³⁵S-labelled particles with rabbit anti-HBs antiserum, followed by polyacrylamide gel electrophoresis and autoradiography, revealed two major bands at 22 kd and 31 kd, and two minor bands at 25 kd and 34 kd (Figure 4).

pHSA receptor activity of HBsAg particles from Vero cells

The adenovirus recombinant AdS(M-B) contained both the S gene and the pre-S2 region. Therefore, we tested the HBsAg particles produced in Vero cells infected by this recombinant for the presence of pHSA receptor activity by means of both hemagglutination of sheep red blood cells coated with pHSA and solid phase RIA. A pHSA binding activity specific for human and not for bovine polymerized albumin was detected (Table II). pHSA receptor activity was not demonstrable in HBsAg particles from Vero cells infected by the recombinant AdS(X-B) which carries only the S gene. This shows that the pre-S2 region of HBV is correctly translated from the recombinant AdS(M-B).

In vivo activity of the recombinant adenovirus expressing HBsAg and pHSA receptor

To determine whether the adenovirus recombinant AdS(M-B) could also direct *in vivo* the synthesis of HBsAg particles carrying the receptor for human polymerized albumin, rabbits were

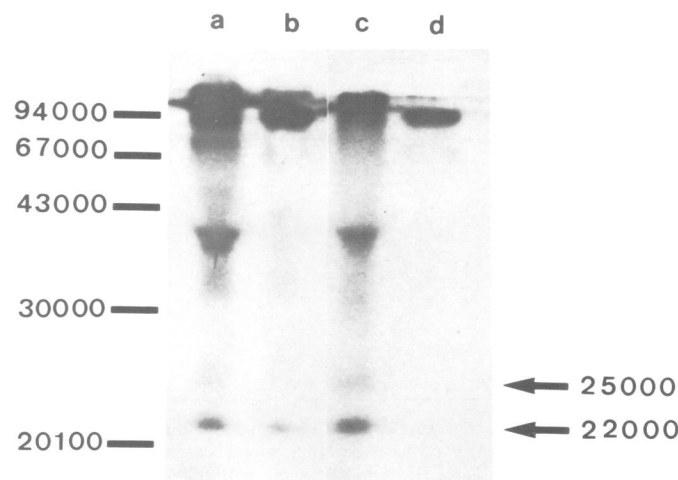


Fig. 4. Autoradiography of ³⁵S-labeled polypeptide material from cell supernatants immunoprecipitated with a rabbit anti-HBs antiserum (see Materials and methods). From left to right: (a) Vero cells infected by the AdS(M-B) recombinant (HBsAg: 50 ng). Incubation with rabbit non-immune serum was not performed. (b) Vero cells infected by the AdS(M-B) recombinant (HBsAg: 50 ng). (c) PLC/PRF/5 cells (HBsAg: 200 ng). (d) Vero cells infected by wild-type Ad5 (HBsAg: negative). Apparent mol. wt. of the polypeptides are given in daltons.

Table II. pHSA receptor activity of HBsAg particles from Vero cells

| | Cell culture supernatant | | | | | | Human serum | | | |
|------|--------------------------|----|----------|----|------|----|--------------------|-----|--------------------|----|
| | AdS(X-B) | | AdS(M-B) | | Mock | | HBsAg + HBeAg + | | Healthy control | |
| | RIA | HA | RIA | HA | RIA | HA | RIA | HA | RIA | HA |
| pHSA | 143 | — | 7512 | 64 | 119 | — | 10 472 | 128 | 65 | — |
| pBSA | 58 | ND | 85 | ND | 77 | ND | 145 | ND | 81 | ND |

Results are expressed in c.p.m for solid phase RIA and in hemagglutination titer (HA), i.e., the highest dilution showing hemagglutination. ND, not determined. AdS(X-B), adenovirus recombinant carrying only the S gene. AdS(M-B), adenovirus recombinant containing both the S gene and the pre-S2 region.

Table III. Anti-HBs response (mIU/ml) in inoculated rabbits

| Weeks | Rabbits | | | | | | | | | |
|-------|---------|---|---|---|---|----|----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 2 | 0 | 0 | 30 | 8 | 6 | 12 | 20 |
| 2 | 2 | 0 | 2 | 0 | 2 | 15 | 20 | 270 | 70 | 85 |
| 3 | 0 | 2 | 0 | 0 | 5 | 18 | 13 | 48 | 15 | 11 |
| 4 | 1 | 0 | 0 | 0 | 2 | 21 | 15 | 27 | 17 | 7 |
| 5 | 0 | 0 | 2 | 0 | 2 | 99 | 52 | 440 | 146 | 136 |
| 6 | 0 | 0 | 0 | 0 | 0 | 78 | 26 | 401 | 151 | 148 |
| 7 | 0 | 0 | 0 | 0 | 0 | 20 | 27 | 325 | 58 | 42 |
| 8 | 0 | 0 | 0 | 0 | 0 | 20 | 40 | 367 | 35 | 6 |

Rabbits were injected i.v. with 10^9 p.f.u. of purified wild-type Ad5 (rabbits 1 and 2) or with 10^9 p.f.u. of purified adenovirus recombinant AdS(M-B) (rabbits 3–10) immediately after the blood drawing in week 0 and week 4.

inoculated i.v. with highly purified preparations of recombinant or wild-type Ad5. Although following inoculation no HBsAg was detected in their sera, five out of eight rabbits inoculated with the recombinant showed the appearance of an anti-HBs titer ranging from 20 to 270 mIU/ml within 15 days (Table III). No anti-

Table IV. Anti-pHSA receptor immune response (reciprocal of the titer)

| Weeks | Rabbits | | | | |
|-------|---------|----|----|----|----|
| | 6 | 7 | 8 | 9 | 10 |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 16 | 2 | 2 | 2 | 2 |
| 2 | 4 | 16 | 16 | 16 | 16 |
| 3 | 4 | 4 | 16 | 8 | 8 |
| 4 | 2 | 2 | 4 | 4 | 4 |
| 5 | 16 | 32 | 32 | 32 | 32 |
| 6 | 16 | 32 | 32 | 16 | 32 |
| 7 | 8 | 8 | 32 | 8 | 16 |
| 8 | 4 | 4 | 8 | 4 | 8 |

Rabbits were infected i.v. with 10^8 p.f.u. of purified adenovirus recombinant AdS(M-B) in week 0 and week 4. Single animals are identified by the same numbers used in Table III. Anti-pHSA receptor activity was expressed as the reciprocal of the highest titer of serum able to give 100% inhibition of hemagglutination. HBsAg particles having a pHSA receptor hemagglutination titer of 1:128 were mixed with an equal volume of serial dilutions of inhibitor sera.

HBs antibodies were detected in rabbits injected with the wild-type adenovirus. After a second i.v. inoculation performed 4 weeks after the first one, a second peak in anti-HBs response was observed reaching 440 mIU/ml in one of the animals. Four weeks after the second injection titers of anti-HBs ranging from 6 to 360 mIU/ml were present. Previous studies indicated that the minimal level of anti-HBs still protective against HBV infection is 10 mIU/ml in man. Furthermore, in view of their relevance in HBV neutralization, we looked for anti-pHSA receptor antibodies in the inoculated rabbits. Anti-pHSA receptor antibodies, as detected by inhibition of the hemagglutination assay, were demonstrated in five out of five anti-HBs positive animals (Table IV).

Characterization of the immunoglobulin class of anti-HBs antibodies demonstrated a mixture of IgM and IgG class-specific antibodies in all the responder animals after the first injection and a predominance of IgG class anti-HBs after the second inoculation (data not shown).

To eliminate the possibility of a contamination of the recombinant adenovirus preparation by HBsAg particles, we looked for their presence by RIA and electron microscopy. Both tests were found to be negative (data not shown).

Discussion

Hepatitis B represents a world-wide health problem since $\sim 200 \times 10^6$ people are chronically infected with HBV and a large number of deaths is due to chronic active hepatitis and cirrhosis. Moreover, a strong association between HBV chronic infection and the development of hepatocellular carcinoma is now clearly demonstrated. The inability to propagate HBV *in vitro* has greatly hampered the development of a vaccine. The present vaccine consists of 22 nm HBsAg particles purified from the serum of chronic carriers. Although the effectiveness and the safety of this vaccine have been widely demonstrated, the availability of human serum and the expense of the vaccine have limited its use in highly endemic areas for mass vaccination campaigns. The use of recombinant DNA technology which allows the production of 22 nm HBsAg particles in eucaryotic cells is, therefore, needed. Presently, HBsAg particles are produced *in vitro* in animal cells (Michel *et al.*, 1984; Patzer *et al.*, 1984) or yeast (Miyahara *et al.*, 1983; Valenzuela *et al.*, 1982) and *in vivo* through the vaccinia virus used as a vector (Smith *et al.*, 1983). Chimpanzees infected

with a vaccinia virus-HBV recombinant were found to be protected against a further HBV challenge (Moss *et al.*, 1984).

The results obtained with the recombinant adenovirus AdS(M-B) showed that adenovirus can be used both *in vitro* to produce HBsAg and *in vivo* as a viral vector capable of eliciting the synthesis of anti-HBs antibodies. The defective nature of the recombinant virus, due to its lack of the E1a region (Jones and Shenk, 1979), could account for the short-lived response in terms of antibodies (probably due to short-term production of antigen) and for the need for a second injection in order to obtain a good immune response. However, anti-HBs titers equivalent to those obtained after the first injection would be sufficient to provide protection against HBV infection.

Although protective against HBV infection, anti-HBs antibodies are unlikely to play a role in recovery from an HBV infection due to their late appearance in serum, weeks or months after the clearance of the complete virion. On the other hand, antibodies against the pHSA receptor which have been shown in serum during acute hepatitis B may have an important role for viral clearance (Alberti *et al.*, 1984; Pontisso *et al.*, 1983b). As a result, it would be useful to use HBsAg particles carrying the pHSA receptor in vaccination programs (Michel *et al.*, 1984). The recombinant adenovirus which is capable of eliciting *in vivo* both anti-HBs and anti-pHSA receptor antibodies would constitute the basis for an efficient live vaccine.

Materials and methods

Cells, cell transfection, virus and plaque assays

The adenovirus-transformed human embryo cell line (line 293-31) was provided by J.C. Nicolas (Hospital Trousseau, Paris) and previously described (Harrison *et al.*, 1977). Monolayer cultures of HeLa and line 293 cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum (FCS). Vero cells and PRF/PLC/5 cells were provided by M.L. Michel (Institut Pasteur, Paris) and were grown in DMEM containing 5% FCS. Transfections were carried out by using the calcium phosphate method (Graham and Van der Eb, 1973). The wild-type Ad5 (H5WT300) was a plaque-purified derivative of a virus stock originally received from T. Shenk (Princeton, NJ). Plaque assays with either virus or DNA were performed on monolayers of 293-31 cells (Harrison *et al.*, 1977).

Materials

Restriction enzymes, T4 DNA ligase and *Escherichia coli* DNA polymerase I (Klenow fragment) were purchased from New England Biolabs and Amersham. [α^{32} -P]dATP (800 Ci/mmol) was obtained from New England Nuclear.

Preparation of plasmid DNAs

Plasmid DNAs were prepared according to the method of Birnboim and Doly (1979), followed by two cesium chloride-ethidium bromide equilibrium gradient centrifugations.

Construction of plasmids

Plasmid pAB1 was constructed as follows: pE1a (TaqI) DNA was digested by *PvuII* and the shortest restriction fragment (nucleotides 452–623) was gel-purified and digested by *HaeIII*. The restriction fragment *PvuII-HaeIII* (nucleotides 452–495) was again gel-purified and finally inserted between the two *PvuII* restriction sites of pE1a (TaqI) (Figure 1A and B).

Plasmid pK4 was constructed by inserting the *BglI-BamHI* restriction fragment of SV40 (nucleotides 5235–2533) between the *HindIII* and *BamHI* restriction sites of pAB1 after filling in the *BglI* and *HindIII* ends with the Klenow fragment (Figure 1C).

Plasmids pK4S(X-B) and pK4S(M-B) were constructed by inserting the restriction fragments *XhoI-BglII* (nucleotides 125–1982) and *MstII-BglII* (nucleotides 3161–1982) of HBV between the *HindIII* and *BamHI* restriction sites of pK4 after filling in the *XhoI*, *MstII*, *BglII* and *BamHI* ends with the Klenow fragment (Figure 1D and E).

Detection and quantitation of HBsAg

Synthesis of HBsAg was detected by using the qualitative RIA AUSRIA II Abbott and quantified using a parallel line assay with an HBsAg standard (20 ng/ml).

Purification of HBsAg

Cell culture supernatants were harvested at different times after infection. HBsAg was purified from pooled supernatants according to Michel *et al.* (1984).

In vivo labelling and immunoprecipitation of HBsAg

Monolayers of Vero cells were infected either with purified wild-type Ad5 or with recombinant adenoviruses. Vero cells, as well as PRF/PLC/5 hepatocarcinoma cells, were maintained for 12 h in methionine-free medium and subsequently labelled twice for 24 h with 150 μ Ci/ml of [35 S]methionine (Amersham, 1.2 mCi/mM). Supernatants of infected cells were collected before and after an additional 24 h chase period. 1 ml fractions of the clarified supernatants were incubated with 10 μ l of rabbit non-immune serum at 4°C for 15 h. Then, 10 mg of pre-swollen Sepharose-coupled protein A (Pharmacia) in 100 μ l of 20 mM Tris-HCl (pH 7.4) was added for 1 h at 4°C. After centrifugation, supernatants were incubated with 10 μ l of rabbit anti-HBs antiserum (Behringwerke) for 4 h at 4°C and then precipitated with protein A-Sepharose as above. The pellet was washed four times in 10 mM Tris HCl (pH 7.4), 150 mM NaCl, 0.5% Nonidet P-40, twice in 125 mM Tris HCl (pH 6.8), and then resuspended in 50 mM Tris HCl (pH 6.8), 15% glycerol, 5% SDS, 0.3 M DTT. Immunoprecipitated proteins were eluted from protein A-Sepharose by boiling for 5 min and, after centrifugation, supernatants were applied to a 12.5% polyacrylamide gel (Laemmli, 1977). After electrophoresis, the gel was fixed in 40% methanol and 10% acetic acid, dried, treated by PPO according to Bonner and Laskey (1974), and exposed to Kodak X-Omat R film at -70°C.

pHSA receptor activity of HBsAg particles

HBsAg receptors for pHSA were determined both by passive hemagglutination of sheep red blood cells (SRBC) coated with pHSA as described by Imai *et al.* (1979) and by a solid-phase RIA as described by Hansson and Purcell (1979) with minor modifications (Pontisso *et al.*, 1983a).

Inoculation of rabbits

New Zealand White female rabbits were inoculated i.v. with highly purified recombinant virus or wild-type virus. Rabbits that showed naturally occurring anti-HBs antibodies were discarded. Antisera were collected at weekly intervals.

Quantitation of antibody response in rabbits inoculated with the recombinant adenovirus expressing HBsAg and pHSA receptor

Levels of antibodies directed against the HBsAg were quantitated in terms of RIA units using the commercially available RIA Kit AUSAB from Abbott. Antibody levels were expressed as International Units [3.5 RIA units are equivalent to 1 milliinternational unit (mIU)]. Anti-polymerized human serum albumin receptor antibodies (anti-pHSA receptor) were detected by hemagglutination inhibition experiments according to Michel *et al.* (1984).

For immunoglobulin class characterization of anti-HBs antibodies, IgG were obtained by ion-exchange chromatography on DE-52 columns (Whatman).

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References

- Aiello, L., Guiloyley, R., Huebner, K. and Weinmann, R. (1979) *Virology*, **94**, 460-469.
- Alberti, A., Pontisso, P., Schiavon, E. and Realdi, G. (1984) *Hepatology*, **4**, 220-226.
- Birnboim, H.C. and Doly, J. (1979) *Nucleic Acids Res.*, **7**, 1513-1523.
- Bonner, W.M. and Laskey, R.A. (1974) *Eur. J. Biochem.*, **46**, 83-88.
- D'Halluin, J.C., Milleville, M. and Boulanger, P.A. (1983) *Gene*, **21**, 165-169.
- Elder, J.T., Spritz, R.A. and Weissman, S.M. (1981) *Annu. Rev. Genet.*, **15**, 295-340.
- Gluzman, Y. (1982) *Eukaryotic Viral Vectors*, published by Cold Spring Harbor Laboratory Press, NY.
- Graham, F. and Van Der Eb, A. (1973) *Virology*, **52**, 456-467.
- Graham, F.L., Smiley, J., Russell, W.C. and Nairn, R. (1977) *J. Gen. Virol.*, **36**, 59-72.
- Hansson, B.G. and Purcell, R.H. (1979) *Infect. Immun.*, **26**, 125-130.
- Harrison, T., Graham, F.L. and Williams, J. (1977) *Virology*, **77**, 319-329.
- Heermann, K.H., Goldmann, V., Schwartz, W., Seyffarth, T., Baumgarten, H. and Gerlich, W.H. (1984) *J. Virol.*, **52**, 396-402.
- Imai, M., Yanase, Y., Nojiri, T., Miyakawa, Y. and Mayumi, M. (1979) *Gastroenterology*, **76**, 241-247.
- Jones, N. and Shenk, T. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3665-3669.
- Laemmli, U.K. (1977) *Nature*, **227**, 680-685.
- Lusky, M. and Botchan, M. (1981) *Nature*, **293**, 79-81.
- Michel, M.L., Pontisso, P., Sobczak, E., Malpice, Y., Streeck, R. and Tiollais, P. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 7708-7712.
- Miyanojara, A., Toh, A., Nozaki, C., Hamada, F., Ohtomo, N. and Matsubara, K. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 1-5.
- Moss, B., Smith, G., Gerin, J., and Purcell, R. (1984) *Nature*, **311**, 67-69.
- Patzner, E., Simonsen, C., Nakamura, G., Hershsberg, R., Gregory, T. and Levinson, D. (1984) in Vyas *et al.* (eds.), *Viral hepatitis and liver disease*, Grune and Stratton Inc., Orlando, FL, pp. 477-485.
- Pontisso, P., Alberti, A., Schiavon, E., Tremolada, F., Bortolotti, F. and Realdi, G. (1983a) *J. Virol. Methods*, **6**, 151-159.
- Pontisso, P., Alberti, A., Bortolotti, F., Realdi, G. (1983b) *Gastroenterology*, **84**, 220-226.
- Rigby, P.W.J. (1982) in Williamson, R. (ed.), *Genetic Engineering*, Vol. 3, Academic Press, London, pp. 83-141.
- Smith, G., Mackett, M. and Moss, B. (1983) *Nature*, **302**, 490-495.
- Spector, D.J. (1983) *Virology*, **130**, 533-538.
- Standing, D.N., Rutter, W.J., Varmus, H.E. and Ganem, D. (1984) *J. Virol.*, **50**, 563-571.
- Tiollais, P., Charnay, P. and Vyas, G.N. (1981) *Science*, **213**, 406-411.
- Toozee, J., (ed.) (1981) *DNA Tumor Viruses*, published by Cold Spring Harbor Laboratory Press, NY.
- Valenzuela, P., Medina, A., Rutter, W., Ammerer, G. and Hall, B. (1982) *Nature*, **298**, 347-350.
- Van Ormondt, H., Maat, J., De Waard, A. and Van Der Eb, A.J. (1978) *Gene*, **4**, 309-328.

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